



STREX Cell Strain Instrument

Catalog # ST-140-04
Serial # 20A-00013

User Manual

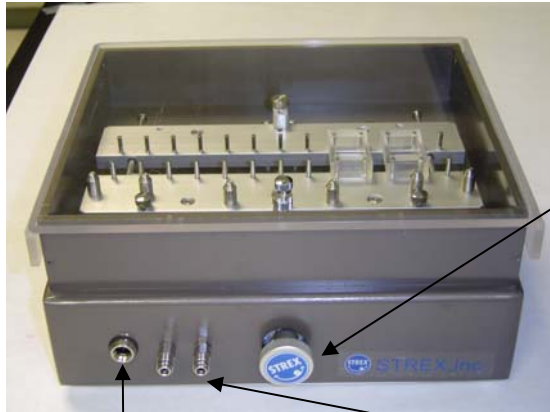
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Section 1: Main Components

Stretch Unit



Chamber length adjusting knob:

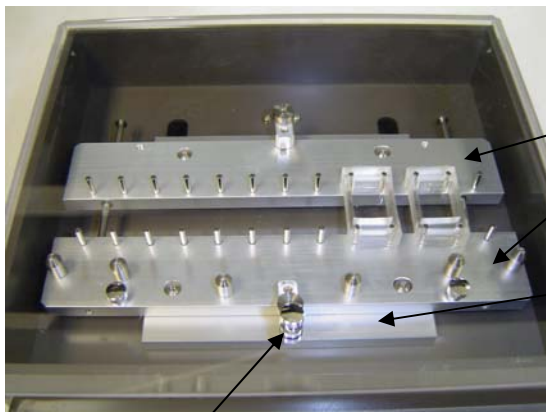
Use the knob to adjust the distance of chamber brackets to maintain tension on the chamber. The bottom of the chamber should be taut.

System coolant inlet and outlet valves:

Be sure to supply cool water when in operation. Failure to do so may lead to overheating inside the incubator or motor burnout.

Connectors:

Use Connector Cable to connect Control Unit to Stretch Unit



Strain Chamber Brackets:

Each chamber is mounted on four pins, two pins on each bracket.

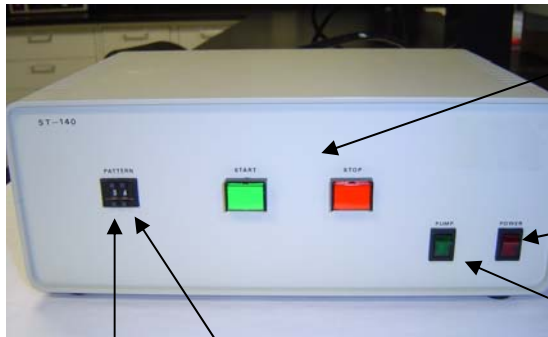
Mounting Stage:

Brackets sit on top of the stage

Alignment Pins:

Holds Strain Chamber Brackets in place

Control Unit Front Panel



Start and Stop Button:
Use to start or stop the stretching action.

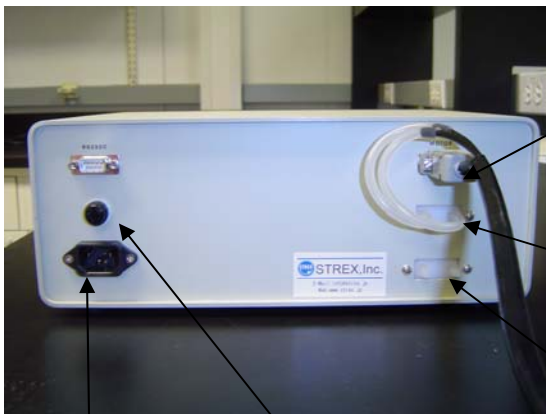
Main Power Switch

Water Pump Switch:
Use to turn on and off the pump that circulates water to cool the motor.

Strain Ratio:
Use upper and lower buttons to increase and decrease stretch ratio

Strain Frequency Selector:
Use upper and lower buttons to adjust cycle frequency.

Control Unit Back Panel



Control Connector:
This cable supplies electricity from the Control Unit to the Stretch Unit.

System coolant inlet & outlet valves:
Be sure to supply cool water when in operation. Failure to do so may lead to overheating inside the incubator or motor burnout.

Fuse case cover

Power Cable Outlet for 110 Volts:
Use the Power Cable to connect the Control Unit to an electrical transformer.

Reservoir coolant inlet & outlet valves:
Attach a large flask or similar type of reservoir filled with cold water.

Electrical Transformer

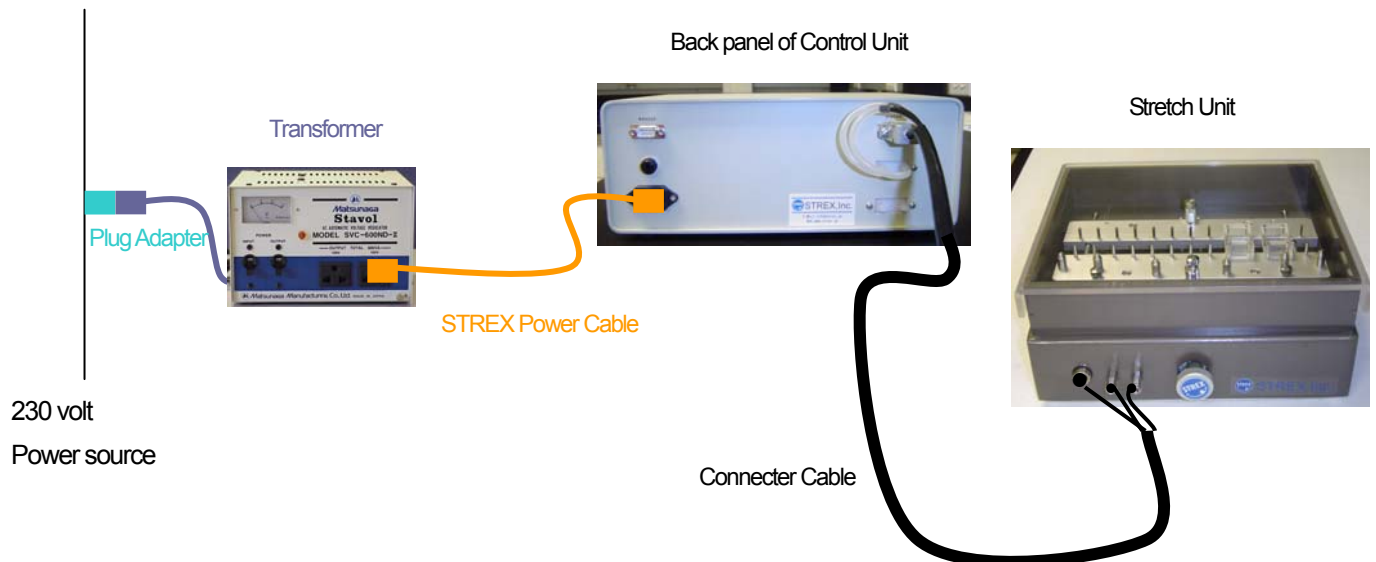


Plug Adapter

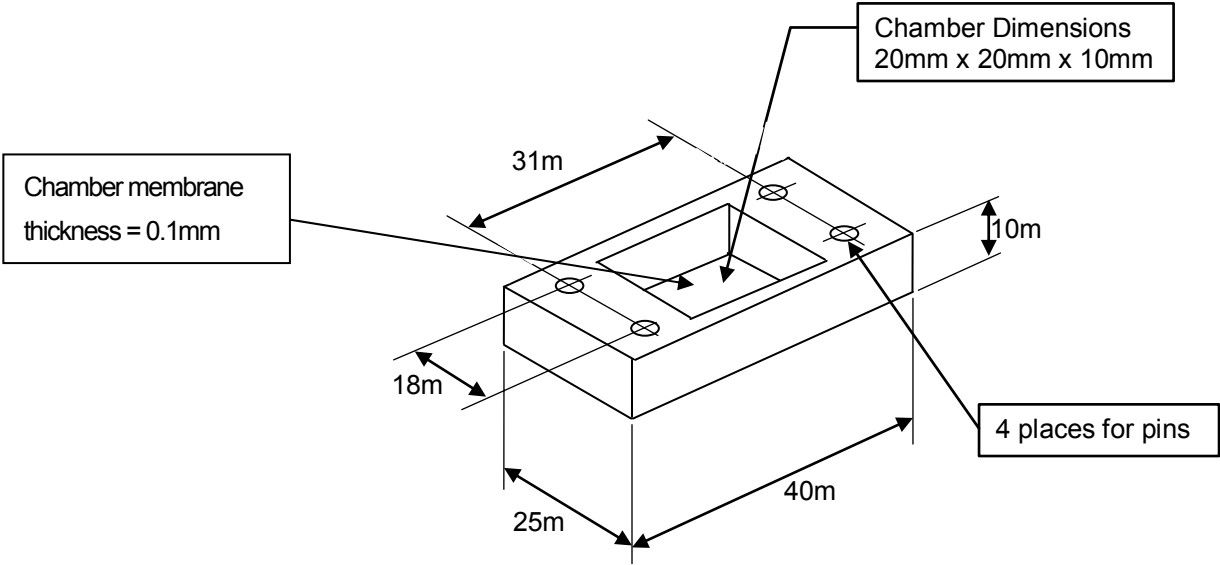


Electrical Transformer
(Also known as Automatic Voltage Regulator)

Electrical Assembly of STREX



Silicone Strain Chamber (ST-CH-04.0)



Section 2: Use of the Cell Strain Instrument

Preparation of the Cell Strain Instrument

Before using the Cell Strain Instrument, sterilize the unit — especially the chamber mounting area — using ethanol-immersed swabs.

System Operation

The STREX Cell Strain System needs to be water cooled when in operation. Without cooling, the maximum run time for the continuous operation of the motor is only 15 minutes. For longer operation, water cooling is essential. Also, please note that, when operating the unit in an incubator, the temperature inside the unit will be elevated so it is particularly important to ensure the cooling system is employed in this environment.

► Set up the Strain and Control Unit

1. Set up the cooling system by using the Connector Cable. The Connector Cable supplies electricity and cold water from the Control Unit to the Stretch Unit. One end of the Cable has 3 plugs of which 2 are identical metal plugs. This end of the Cable attaches to the front of the Stretch Unit. The 2 identical metal plugs are plugged into the System Coolant Inlet and Outlet Valves (see Section 1). To release the metal plugs from the Stretch Unit, push backward on the outer metal rings around the valves. The single, larger black plug supplies electricity to the Stretch Unit – this plug has to be in the correct orientation to fit properly. Improper orientation will break the outlet.

The other end of the Connector Cable has 2 exposed latex tubes which fit onto the System Coolant Inlet and Outlet Valves on the back panel of the Control Unit. The Cable's square shaped plug fits the motor outlet just above the coolant valves.

To circulate cool water throughout the system, use latex tubing to attach a large reservoir of cold water to the Reservoir Coolant Inlet and Outlet Valves on the back panel of the Control Unit. The water in the reservoir can be kept cool by placing the reservoir on ice.

2. The Control Unit was made for 110 Volts therefore the Control Unit must be connected to an electrical transformer (refer to section 1) by the Power Cable.
3. Please read the Stavol instructions before using the transformer.
 - A) Use the plug adapter to connect the transformer to your 230 Volt power source
 - B) Flip the transformer's Input Switch to the ON position
 - C) When the orange light is ON and the volt meter indicates 100 Volts, then the transformer is ready to use.
 - D) Connect the STREX Control Unit to the transformer using the Power Cable. The Power Cable connects from the back of the Control Unit to the Output

plugs in the transformer.

E) Flip the transformer's Output Switch to the ON position.

IMPORTANT: When turning OFF the STREX instrument, follow the steps in the order listed below.

- ① Turn OFF the STREX Control Unit
 - ② Flip the Output Switch to the OFF position
 - ③ Flip the Input Switch to the OFF position.
4. Turn on the Main Power Switch and Water Pump Switch on the Control Unit. The Switches will light up.
 5. Press the START button to ensure that the strain chamber bracket moves correctly. The Start Button light will flash when the instrument is stretching.
 6. Press the STOP button to ensure that the strain chamber bracket stops moving. The Stop Button will light up.
 7. Make sure that the water pump is pumping water from the reservoir and through the Control and Stretch Unit.
 8. Turn off Main Power and Water Pump Switches.

► Start Cell Stretching

1. **The Power Switch must be OFF to freely rotate the Chamber Length Adjustment Knob.** Silicone chambers containing cultured cells are attached to the Stretch Unit by inserting the 4 pins into the corners of the chamber. Rotate the Adjustment Knob clockwise until the bottom of the chamber is taut.
2. Select the stretch parameters by changing the Stretch Pattern Buttons. Refer to Section 3 for stretch parameters.
3. **Do not change the Strain Ratio or Strain Frequency Selector Buttons during the operation of a stretch cycle. Press the Stop Button and wait for the last cycle to complete and the chamber returns to its starting position before changing stretch parameters.** Changing parameters during a stretch cycle may damage the motors.
4. Push the Start Button to start the stretch unit.
5. After 5 minutes, stop the stretching and check the condition of the cells. If the cells have not detached, proceed with your experiment. If the cells are detached, the adhesion matrix coating was probably insufficient. Recoat the chambers.

Culturing Cells in the Silicone Chambers

1. Seed cells at the appropriate concentrations in the freshly coated chambers.

Important: It is critical to not over expose the cells to dissociation enzymes. Cells should be treated in the same manner (type, concentration of enzyme, temperature, and exposure time) for all experiments.

Important: Cells should not be cultured at a high cell density in the chambers. For example, epithelial cells often form a cell-sheet and the cell-cell adhesion seems to be stronger than a cell-surface adhesion. When this happens cells may detach from the chamber. Additionally, cultures that are grown over a week in the chambers may detach.

2. After overnight incubation, inspect cells under a microscope to ensure that they adhered to the chamber.

Preparation of Silicone Chambers

Before using the chambers, they should be sterilized then coated with a cell adhesion matrix. The coating procedures below can be adapted for use with other matrices, such as elastin, pronectin, and laminin.

Sterilize chambers in an autoclave for 20 minutes at 121°C. The silicone chambers can withstand temperatures up to 180°C. Use of an autoclave is preferable. However, if an autoclave is not available, the chambers may be sterilized by submerging in 70% ethanol, rinsing with water, then drying in a sterile environment.

Place the sterile silicone chambers in a Petri dish in preparation for coating.

► Fibronectin Coating

Preparation of fibronectin solution:

1. Dilute human or bovine fibronectin to a final concentration of 50 to 100 ug/ml in Phosphate Buffered Saline (PBS)

Coating with fibronectin solution:

1. Pour 3 ml of the fibronectin solution into each strain chamber
2. Incubate at 37°C for more than 30 minutes
3. Aspirate the fibronectin solution. If coating is successful, water will not be repelled after removing the fibronectin solution.
4. The liquid solution can be used to coat 3 or 4 chambers before discarding.

PBS (per liter):

NaCl	8.00 g
KCl	0.20 g
Na ₂ HP ₄ (anhyd.)	1.15 g
KH ₂ PO ₄ (anhyd.)	0.20 g

Note: Dulbecco's PBS in powder form for tissue culture applications is also commercially available.

► Gelatin Coating

Preparation of gelatin solution:

1. Add gelatin powder to PBS at a concentration of 2%
2. Autoclave the mixture to dissolve and sterilize

Coating with gelatin solution:

1. Pour 3 ml of the gelatin solution into each strain chamber
2. Incubate at 37°C for more than 30 minutes
3. Aspirate the gelatin solution. If coating is successful, water will not be repelled after removing the gelatin solution.
4. The liquid solution can be used to coat 3 or 4 chambers before discarding.

► Collagen Coating (Cellmatrix 1-C, P, Type 3 or 4)

Preparation of collagen solution:

1. Combine 1 part collagen to 10 parts HCL, pH 3, in a sterile tube

Coating with collagen solution:

1. Coat chamber with a thin layer
2. Aspirate excess
3. Dry in biological safety cabinet at 25°C or below. The chamber can be stored at the same temperature.
4. Wash the chamber twice with culture medium.
5. If coating is successful, water will not be repelled.

The PDMS (silicone) chambers are very hydrophobic with two methyl-bases on the surface. Cells adhere to the fibronectin or collagen coated chambers via integrins. This form of cell adhesion is very different compared to attachment of cells to plastic or glass dishes where the surface of plastic or glass is charged, resulting in non-specific binding. If the cells are having difficulty attaching to the coated chamber or easily detached upon stretching, treat the stretch chamber with a higher concentration of fibronectin or collagen, for overnight.

Section 3: Strain Parameters (Stretch)

RIGHT Digit: Strain Ratio

Digit	Degree of stretch	Distance
0	2%	0.4 mm
1	4%	0.8 mm
2	5%	1.0 mm
3	8%	1.6 mm
4	10%	2.0 mm
5	15%	3.0 mm
6	16%	3.2 mm
7	20%	4.0 mm

LEFT Digit: Strain Frequency Selector

Digit	Program	Description*
0	1 cycle/100 seconds Square wave	Hold 49 sec – stretch 1 sec – hold 49 sec – contract 1 sec – repeat
1	1 cycle/minute Square wave	Hold 29 sec – stretch 1 sec – hold 29 sec – contract 1 sec – repeat
2	10 cycles/minute Square wave	Hold 2 sec – stretch 1 sec – hold 2 sec – contract 1 sec – repeat
3	30 cycles/minute Sine wave	Stretch 1 sec – contract 1 sec – repeat
4	60 cycles/minute Sine wave	Stretch 0.5 sec – contract 0.5 sec – repeat
5	1 cycle	Stretch 1 sec – hold 3 hours – contract 1 sec
6	Static	Stretch 1 sec – hold indefinitely
7	Static	Stretch 0.5 sec – hold indefinitely

*sec is the abbreviation for seconds

Section 4: FAQ

Q1: What are the characteristics of the silicone chamber?

A1: The strain chamber is made from silicone elastomer consisting of polydiethylsiloxane as its major component. The chamber surface is strongly hydrophobic and cells have difficulty attaching to it; therefore, the chamber surface should be coated with an extra-cellular matrix like fibronectin, collagen, laminin, or gelatin before cultivation.

Q2: Cell attachment on the stretch chamber is not consistent.

A2: There may be wrinkles or bubbles on the bottom surface of the strain chamber when seeding cells. Although the chamber is carefully made not to have wrinkles on it, some products might have little wrinkles due to its thin structure. We recommend the following steps. Place a small volume of ethanol in a Petri dish that is large enough to hold the strain chamber. Gently place the chamber in the culture dish starting at one edge and moving toward the opposite edge of the chamber to remove air bubbles between the dish and chamber. Allow the ethanol to evaporate before spreading your cell suspension in the chamber.

Q3: Cell attachment on the stretch chamber was confirmed by microscopy. But the cells detached from the chamber surface after stretching the cells.

A3: Try seeding your chambers at a lower concentration of cells. Over-confluent cells generally adhere to neighboring cells rather than to the base matrix (dish surface). When an excess amount of cells are put in a culture dish, the cells connect to each other after growth. Such behavior of the over-confluent cells, which are often observed in normal culture dishes, is even worse in the strain chamber.

A second possibility for cell detachment is that the cells were damaged by enzyme treatment such as trypsin before seeding. The damaged cells sometime attach to surfaces by non-specific binding and are not specifically bound to the extra-cellular matrix coating on the chamber; therefore, time, concentration, and temperature for the enzyme treatment should be optimized to reduce cell damage.

A third possibility is insufficient coating of the chamber preventing the cells from attaching to the chamber. Longer coating time is recommended. Some researchers coated the chamber with two or more kinds of the extra-cellular matrix materials to increase binding effectiveness.

Q4: How long can cells be stretched?

A4: The duration depends on cell strain and condition. In general, two weeks cultivation in the incubator is possible with culture media changes every 2-3 days. It is also important to monitor reservoir coolant for the instrument motor. Insufficient amount of the cooling water may increase the temperature of the motor resulting in killing of cells and /or burning out the motor.

Q5: How can I obtain protein or mRNA samples from the cells attached to the silicone membrane?

A5: (1) Proteins for Western blotting: Wash the cells once with PBS. Add SDS-PAGE sample loading dye directly into the chamber, and collect the cell extract by using a cell scraper.

(2) Proteins for Immunoprecipitation: Wash the cells once with PBS. Add cell extract buffer directly into the chamber, and collect the cell extract by using a cell scraper.

(3) RNA: Wash the cells once with PBS (for RNA preparation). Add RNA extraction buffer directly into the chamber, and collect the cell extract by using a cell scraper.

Q6: I want to use recombinant cells for an experiment.

A6: Direct transfection of cells in the strain chamber may be possible. However, transfection itself may damage the cells, which may make getting clear image data difficult. We recommend performing the transfection in a normal culture dish then transferring the recombinant cells into the strain chamber.

Q7: Cells seem to be crowded in the center of the chamber instead of being uniformly distributed throughout the chamber.

A7: Vibration from the incubator may disrupt the distribution of the cells. We recommend gently rocking the chamber 15 minutes after seeding your cells.

Section 5: References

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Section 6: Safety Instructions

Please read this section carefully before using the instrument. Items in this section alert the user to operational dangers that, if not followed, may damage the instrument or, more significantly, result in serious injury or death of the user. To ensure safe operation of the instrument, it is therefore imperative that you follow these instructions carefully.

Power cable

To avoid possible short circuit, shock, or fire

- Only use the power cable provided with the Cell Strain Instrument.
- Do not touch the cable with wet hands.
- Do not use the machine with other voltage than that specified. In some cases, a transformer may be used for compatibility. Inappropriate current may result in the machine overheating, short-circuiting, and/or fire may occur.
- Do not staple around the power cable.
- Do not bend the cable or place heavy objects on it.
- When pulling a connector from an outlet, pull to disconnect gently by holding its plug, not the cable.
- Do not plug many objects into a single electrical outlet since it may cause fire.
- If you are using an extension cord, ensure it can withstand the total current to be used.
- Disconnect power from the unit when it is not in use.
- Connect the instrument to a power-surge protected outlet.

Installation Location and Environment

- Keep the instrument on a stable, leveled floor or a table, secure from vibrations. Be sure you have enough space.
- Do not store the instrument in a humid or dusty place. Over time, excessive humidity or dust may cause deterioration that can result in an electrical short-circuit and possibly fire.
- Do not use the machine in a place where the temperature is excessively high. Do not place and run the machine near a heater or in a place being exposed to a direct sunlight.
- To avoid possibly explosion, never place and run the instrument nearby the presence of flammable solid substance, liquid, or gas. It may cause explosion or fire.
- Use the machine in well lit conditions.
- Do not use the machine outdoors in direct sunlight or rain, which may cause overheating or short circuit.

Operational Concerns

- Please make sure to read the manual prior to running the unit. Those who are not familiar with the machine should not operate it.
- Do not put your hand close to mechanical parts or alike while the unit is running.
- Do not put any foreign substances inside the machine. Water, metal, or paper in motor area, may cause fire or electrical shock.
- Do not make any attempt to disassemble or modify the machine. Do not remove the cover in an attempt to touch the mechanism inside, which may cause you an electrical shock.
- Please refrain from modifying the machine without our permission, you may be shocked or injured. If you do attempt to modify the machine, the warranty on the unit is void and we will not be responsible for any performance deterioration or unit malfunction.
- In the case of any abnormal sound, smell, or smoke, disconnect the power immediately and contact B-Bridge International.
- Do not run the machine overloaded.
- Be cautious as to your clothing and hair when operating the instrument. Baggy clothing, neckties, necklaces, etc., can get tangled in moving parts of the unit. Take appropriate precautions to prevent this occurrence.
- Keep the machine clean and periodically inspect the instrument for excessive wear or damage. Contact B-Bridge if you have any concerns.

Section 7: Warranty

1. The warranty is for one year, commencing the date the customer receives the product and includes the instrument casing, non-wearable parts, as well as, the motor and bearings. The cell culture chambers are considered consumables, B-Bridge International, Inc. is responsible for repair or replacement of chambers, only if they are received and found defective.
2. The warranty does not cover damage to the instrument that is a result of the following circumstances:
 - ① Damage caused by dropping, or other impact.
 - ② Damage caused by inappropriate operation of the instrument.
 - ③ Damage resulting from an attempted repair or modification of the instrument by the user.
 - ④ Damage caused by unavoidable external causes such as earthquakes, lightening, fire, flood, gas leak, power surges, or other acts of providence.
3. B-Bridge International, Inc. is free from any responsibility for effects or loss or damages arising from the result of the machine operation.

This warranty assures that B-Bridge International, Inc. will repair our product free of charge as stipulated in our warranty policy. Any shipping charges will be born by buyer.

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